Evaluation of Off-Flavor Development in Alpine Cheese Using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS)

Elana Berusch, Kaitlyn Taylor, and W. James Harper

Abstract

A manufacturer of Alpine cheese has found that within three weeks of removing their product from vacuum packaging, off-flavors start to develop in the cheese. It was speculated that the development of these off-flavors was caused by lipid oxidation. The objective of this study was twofold: determine if the flavor change is, in fact, caused by lipid oxidation, and if it is not, find the agent causing the flavor profile to change. Both goals were met by using selected ion flow tube-mass spectrometry (SIFT-MS) to evaluate two different lots of Alpine cheese, #153 and #160. SIFT-MS is a direct mass spectrometric technique used to quantify volatile compounds in the headspace of a sample in real time. To prepare the samples, both cheeses were divided in half and grated – one part vacuum sealed and the other part exposed to oxygen. Over the course of 56 days, both the samples exposed to oxygen and the vacuum-packed samples were examined for development of off-flavors using SIFT-MS for cheeses #153 and #160. Concentrations of thirty-two compounds in the cheeses, including alcohols, aldehydes, ketones, esters, sulfur compounds, and pyrazines, were analyzed. The results showed that eight compounds, which changed in concentration over the testing period in samples exposed to oxygen, were derived from degradation of amino acids and lipids. This suggests that the off-flavors produced in the Alpine cheeses are due to amino acid degradation as well as lipid oxidation. The compounds that underwent significant concentration changes, however, varied between cheese #153 and #160. In addition, the impact of time and oxygen on cheese #160 appears to be far greater than that on cheese #153. Further studies will be done to narrow down the causes of the changes in the Alpine cheese flavor profile and to determine ways to prevent the development of these off-flavors.

Introduction

Alpine cheese is made from the milk of grass-fed cows, and the nature of the cows' diet causes the cheese to have especially high concentrations of ω-3 fatty acids, especially alpha-linolenic acid, when compared to other cheeses. These ω-3 fatty acids are especially susceptible to lipid oxidation, and such oxidation can contribute an off-flavor to the Alpine cheese (Hausworth and others 2003).

In order to identify the off-flavor, Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) in combination with odor activity values (OAVs) was used to identify important volatile compounds found in the headspace of Alpine Cheese. SIFT-MS is a direct mass spectrometric technique based on chemical ionization of a gas sample using selected precursor reagent positive ions (Spanel and Smith 1999). SIFT-MS makes it possible to measure volatile organic compound headspace concentrations in parts-per-trillion in real time.

Odor activity value (OAV) is the compound concentration divided by its odor recognition threshold in air. Compound concentrations alone can indicate physical changes within a sample but do not indicate the impact of a given compound on the overall flavor profile. The OAV is an indicator of

how much impact the compound in question actually has on the overall flavor profile and was therefore a value more useful than compound concentration in this study. Compounds with an OAV $\geq$1 are above the recognition threshold and therefore considered to have "high impact" on flavor (Preininger and Grosch 1994).

Materials and Methods

Lot #153 and Lot #160 were used in this study. Both lots are of the Alpine variety and experiencing off-flavor development within three weeks of removal from packaging. The same methods of preparation and evaluation were carried out on both lots. First, the cheese samples were divided in half. Both halves were grated. One half of the grated cheese was vacuum-packed and the other half was sealed in a bag containing air. Both packaged samples were stored in a refrigerator at 4^{o}C for the duration of the testing period. Before evaluation using SIFT-MS, the cheese samples were removed from the refrigerator. Sample bottles were filled with five grams of cheese, and these bottles were then equilibrated at 40^{o}C for one hour. Using SIFT-MS, 32 volatile compounds were measured in real time. Cheese samples were analyzed at days 0, 7, 14, 21 and 56 to track changing flavor profiles. The collected data were analyzed using one-way analysis of variance (ANOVA) and Pirouette programs.

Results

The concentrations of 32 compounds in lots #160 and #153 were measured in parts-per-billion by SIFT-MS testing over the course of 56 days. These 32 compounds included alcohols, aldehydes, ketones, esters, sulfur compounds, and pyrazines. Since odor activity values give more of an indication of the impact of a particular compound on overall flavor, these concentrations were converted into OAVs. No significant change occurred in the cheeses that were stored in vacuum-packed conditions. In the oxygen-exposed cheese samples, it was found that eight of the 32 compounds which changed in concentration – and therefore OAV - over the testing period were derived from degradation of amino acids and lipids. This suggests that the off-flavors produced in the Alpine cheeses are due to amino acid degradation as well as lipid oxidation.

Table 1 shows that out of the eight targeted compounds in Lot #160, four underwent significant OAV change over time. These compounds – dimethyl sulfide, ethyl methyl sulfide, methional, and methyl mercaptan – are all products of amino acid degradation. Since the four compounds associated with lipid oxidation showed no significant change over time, it was determined that the Alpine flavor degradation was due to amino acid breakdown, not lipid oxidation. Overall, the average OAVs of methyl mercaptan and dimethyl sulfide decreased while the average OAVs of ethyl methyl sulfide and methional increased.

Table 2 shows that the significantly changing compounds in Lot #153 are the same four amino acid-related compounds as in Lot #160. The concentrations and OAVs of dimethyl sulfide and methyl mercaptan decrease while those of ethyl methyl sulfide and methional increase.

Table 1. Changes in Odor Activity Values (OAVs) in Alpine cheese #160 over 56 days while exposed to oxygen.

Compound	Odor Threshold (ppb)	Average Odor Activity Value				
		Day 0	Day 7	Day 14	Day 21	Day 56
1-octen-3-ol	9.15	3.14[a]	4.33[a]	3.93[a]	2.22[a]	0.99[a]
1-octen-3-one	0.12	80.64[a]	118.22[a]	95.45[a]	82.12[a]	46.80[a]
(E)-2-heptenal	610.33	0.007[a]	0.015[a]	0.014[a]	0.016[a]	0.008[a]
(E)-2-hexenal	448.43	0.008[a]	0.016[a]	0.016[a]	0.018[a]	0.007[a]
(E)-2-nonenal	0.19	11.32[a]	20.71[a]	21.36[a]	24.24[a]	10.88[a]
Dimethyl sulfide	6.26	4.89[ab]	7.37[a]	4.39[ab]	2.11[ab]	0.51[b]
Ethyl methyl sulfide	39.81	1.42[b]	3.83[ab]	5.00[a]	4.52[ab]	4.57[ab]
Methional	0.01	1736.82[b]	5178.17[ab]	6451.73[a]	5745.10[ab]	4504.41[ab]
Methyl mercaptan	1.27	105.74[ab]	202.73[a]	179.86[ab]	128.08[ab]	49.33[b]

[a,b] Different letters indicated significant differences over time.

Purple indicates lipid oxidation compounds. Blue indicates amino acid degradation compounds

Table 2. Changes in Odor Activity Values (OAVs) in Alpine cheese #153 over 56 days while exposed to oxygen.

Compound	Odor Threshold (ppb)	Average Odor Activity Value				
		Day 0	Day 7	Day 14	Day 21	Day 56
1-octen-3-ol	9.15	1.77[a]	2.02[a]	2.73[a]	1.41[a]	0.89[a]
1-octen-3-one	0.12	65.99[a]	94.96[a]	126.29[a]	73.35[a]	54.26[a]
(E)-2-heptenal	610.33	0.007[a]	0.010[a]	0.014[a]	0.014[a]	0.010[a]
(E)-2-hexenal	448.43	0.006[a]	0.009[a]	0.015[a]	0.015[a]	0.008[a]
(E)-2-nonenal	0.19	9.31[a]	12.80[a]	19.18[a]	14.82[a]	13.42[a]
Dimethyl sulfide	6.26	2.30[ab]	3.14[a]	1.65[ab]	0.59[ab]	0.26[b]
Ethyl methyl sulfide	39.81	1.12[b]	2.74[b]	3.07[b]	2.24[b]	19.39[a]
Methional	0.01	1544.34[b]	2038.48[b]	2253.64[b]	2580.18[b]	11610.35[a]
Methyl mercaptan	1.27	100.66[ab]	116.80[a]	94.59[ab]	51.51[ab]	14.84[b]

[a,b] Different letters indicated significant differences over time.

Purple indicates lipid oxidation compounds. Blue indicates amino acid degradation compounds

Figures 1-4 depict the changing OAVs of the four significant compounds in both lots of Alpine cheese. It can be seen in Figures 1 and 2 that the average OAVs of dimethyl sulfide and methyl mercaptan in both #160 and #153 spike at Day 7 but then sharply decrease. Conversely, Figures 3 and 4 show that the OAVs of ethyl methyl sulfide and methional remain relatively constant in Lot #153 from Day 0 to Day 21, but then drastically spike at Day 56. An increase in both compounds is also shown in Lot #160, although this increase was found to be more gradual.

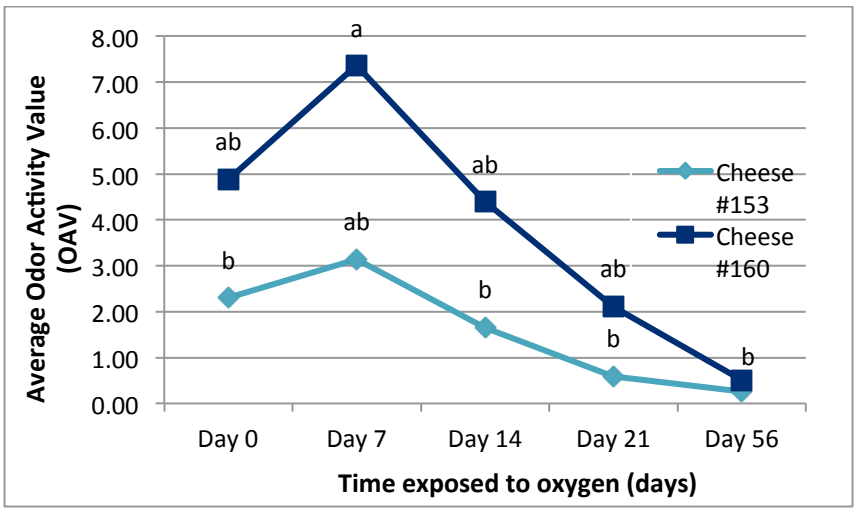

Figure 1. Average OAVs of dimethyl sulfide over time in oxygen-exposed Lots #153 and #160.

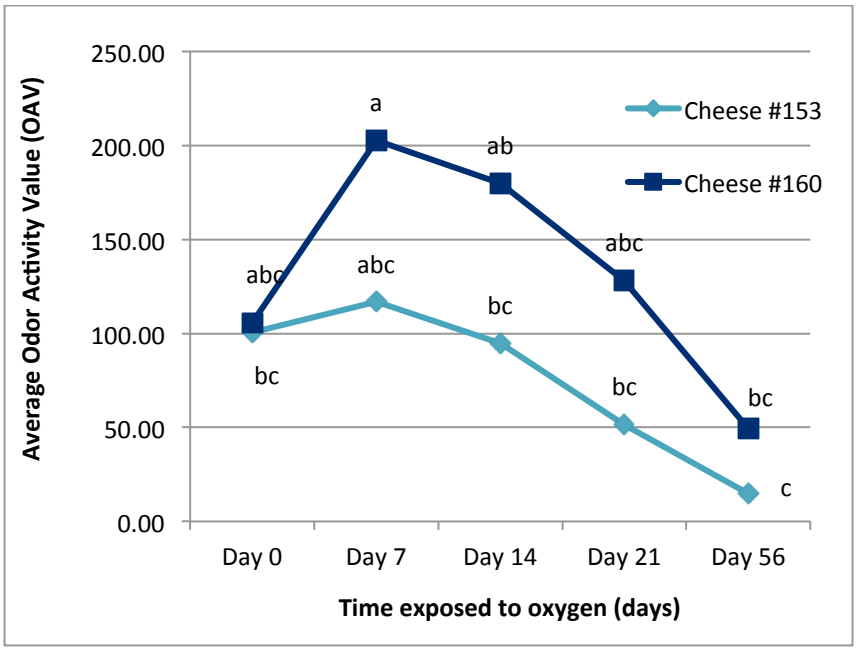

Figure 2. Average OAVs of methyl mercaptan over time in oxygen-exposed Lots #153 and #160.

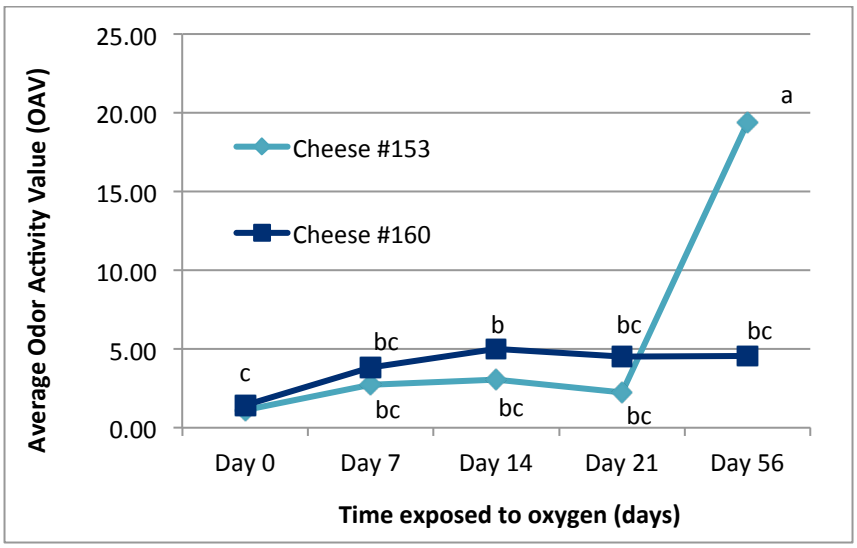

Figure 3. Average OAVs of ethyl methyl sulfide over time in oxygen-exposed Lots #153 and #160.

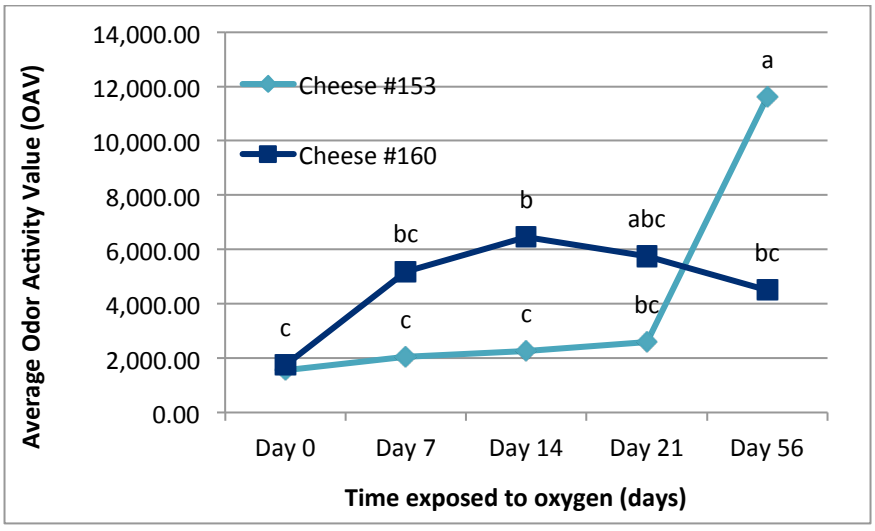

Figure 4. Average OAVs of methional over time in oxygen-exposed Lots #160 and #153.

The increase in ethyl methyl sulfide and methional OAVs alongside decreasing dimethyl sulfide and methyl mercaptan OAVs can be explained by the biochemical degradation pathway of the amino acid methionine. The methionine breakdown pathway is shown in Figure 5. The amino acid initially degrades into methional and methyl mercaptan. This explains the increase in methyl mercaptan and methional in Lots #153 and #160 between Days 0 and 7. Methional is a final product, which is why the OAVs of methional continue increasing as methionine breaks down. Methyl mercaptan, however, continues degrading into ethyl methyl sulfide and dimethyl sulfide. This is why both ethyl methyl sulfide and dimethyl sulfide are shown in increase in OAV in the same week that methyl mercaptan OAVs begin to decrease. Dimethyl sulfide continues degrading into trimethyl sulfide and further, which explains the sharp decrease in the compound's OAVs over time.

Methional and ethyl methyl sulfide are both final breakdown products of methionine, so it was expected that the OAVs of these compounds would gradually increase and then level off. However, a sharp spike in OAV is shown for both compounds in Lot #153 at Day 56. Further study is required to determine why this spike occurs.

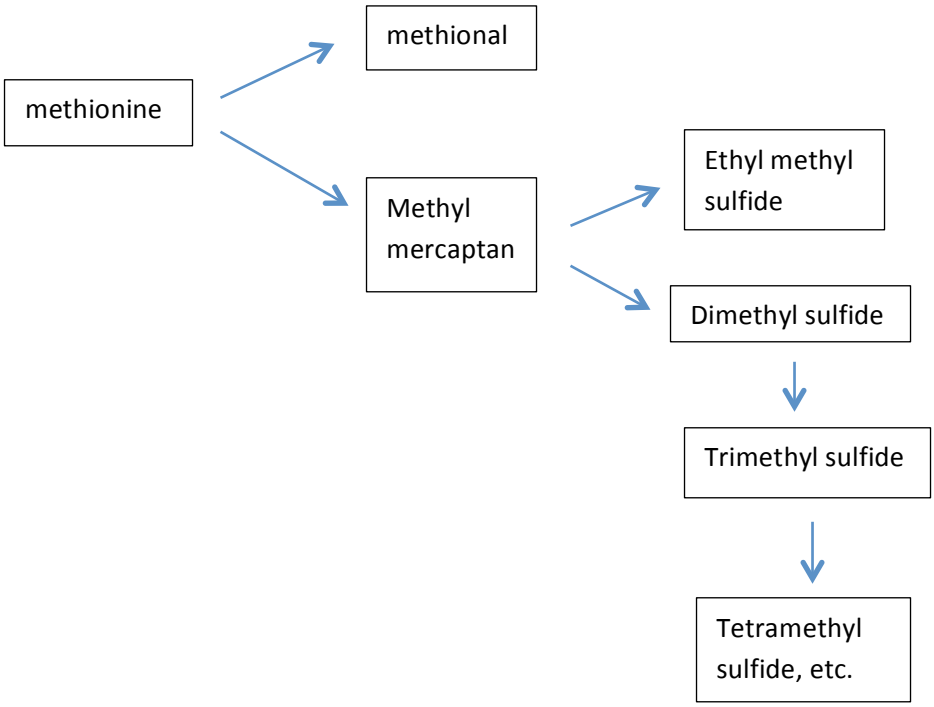

Figure 5. Methionine breakdown pathway.

Conclusion and Further Study

Through OAV analysis of compounds commonly associated with lipid oxidation, it was determined that lipid oxidation is not responsible for the changing flavor profiles of Cheeses #153 and #160. Since consumer complaints reported that Cheeses #153 and #160 developed the same off-flavor over time, compounds that underwent the same OAV changes in both cheeses over time were examined. It was found that four compounds had significant changes in OAV over time in both Cheese #153 and Cheese #160. These compounds were methional, methyl mercaptan, ethyl methyl sulfide, and dimethyl sulfide, all of which are derived from the degradation of the amino acid methionine.

Alpine cheese samples containing tocopherol, an antioxidant, are currently being studied to determine whether or not the presence of tocopherol in the cheese prevents the development of off-flavors. In the future, the cheese manufacturer may benefit from studying the causes of methionine degradation and relating those causes to factors of Alpine cheese production. Sensory studies may also help narrow down the particular off-flavor that is forming over time.

References

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